



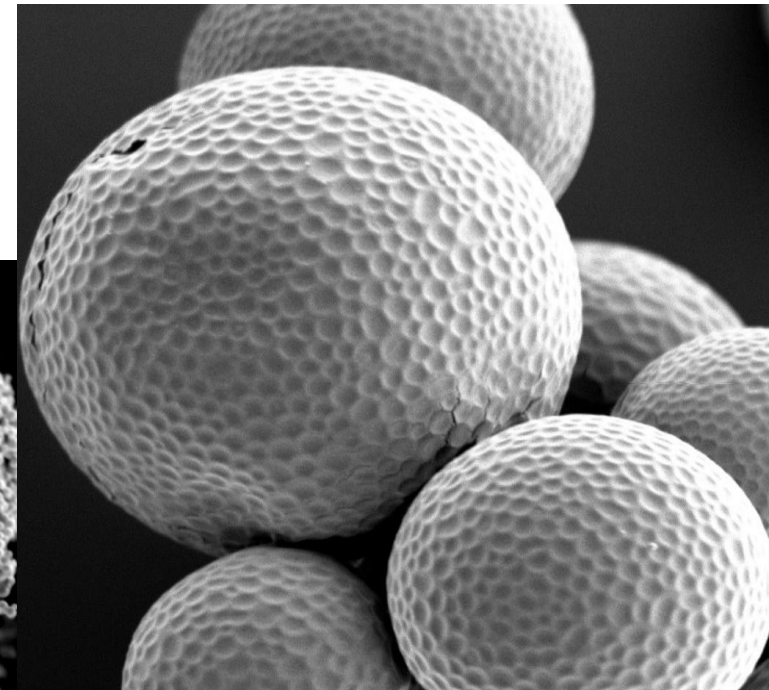
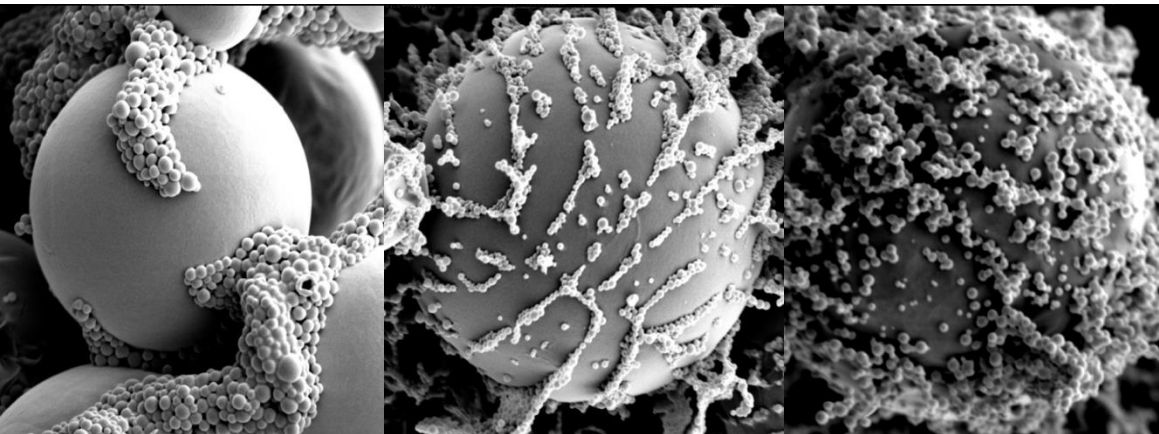
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# NANOTECHNOLOGY FOR FABRICATION OF NANOPARTICLES AND NANOEMULSION

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# Outline

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- 3 concepts
  - ✓ Design of Dosage Forms Using Nanotechnology
  - ✓ Biopharmaceutic & Biopharmaceutic Classification System
  - ✓ Fusion concept between contemporary and prophetic medicine
  - ✓ Summary of Discussion & Conclusion
- 2 strategies
  - ✓ Fabrication of plasmid DNA- Nigella sativa oil nanoparticles
  - ✓ Fabrication of Doxycycline – Nigella sativa nanoemulsion
  - ✓ Summary of Discussion & Conclusion





# Design of Dosage Form

**What is Nanotechnology in global context –**  
based on ISO Technical meeting 2010:

**“application of scientific knowledge to manipulate and control matter in the nanoscale range to make use of size- and structure-dependent properties and phenomena distinct from those at smaller or larger scales”.**

*(Full acceptance and use of the ISO definition in the environmental, health, and safety (EHS) community has not yet been resolved) (Lovestam et al. 2010)*







# Design of Dosage Form

What is nanotechnology in the context of Drug Delivery System –

“science/engineering or technology that allows any raw materials (e.g small MW drugs, natural products, macromolecules) to become NANO-sized particles/crystals/powders”.

**What is NANO-sized particles =**  
**NANOparticles → less than 300 to 100 nm (the size is chosen based on safety profile)**

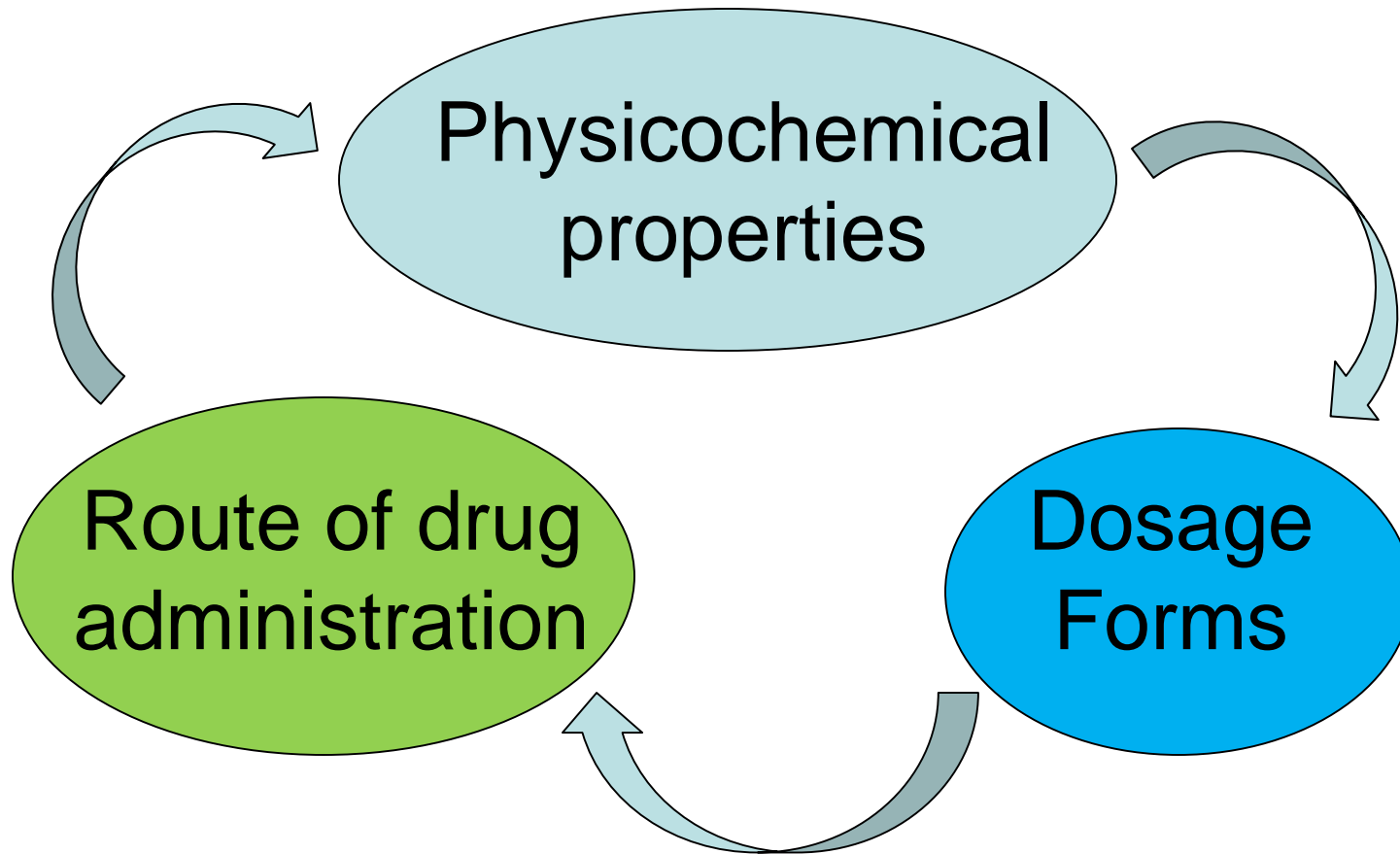




# Concept #2.1

## Biopharmaceutic

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Rate & extent of absorption

= BIOAVAILABILITY





# Concept #2.2 BCS

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## BCS – Biopharmaceutics Classification System

Class I – High Permeability & High Solubility

HIGHEST  
ORAL F

Class II – High Permeability & Low Solubility

HIGHER  
ORAL F

Class III – Low Permeability & High Solubility

Class IV – Low Permeability & Low Solubility





# CONCEPT #3 FUSION

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**Contemporary medicine**

**+**

**Prophetic medicine**



Novel  
Dosage  
Form

**Fusion medicine**



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# FUSION CONCEPT

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Novel  
Dosage  
Form

## Nanoparticles

- DNA + Nigella sativa oil

Novel  
Dosage  
Form

## Nanoemulsion

- Doxycycline hyclate + Nigella sativa oil

Contemporary + prophetic/herbal



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Strategy #1

Fabrication of nanoparticles

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# Fabrication of Plasmid DNA + *Nigella sativa* Nanoparticles



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# Strategy #1 Fabrication of nanoparticles

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## Physicochemical properties

### Plasmid DNA

- labile
- Negatively charged
- ~5 kilo base pair
- Water-soluble / hydrophilic
- Precipitate in ethanol
- Size ~1-2 nm





# Strategy #1 Fabrication of nanoparticles

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## Physicochemical properties **Nigella sativa oil**

- Has multiple compounds (~50% fatty acids + ~40% sterols + 10% tocopherol)
- Lypophilic / oil
- Soluble in organic solvent
- Surface tension ~30mN/m

*Water surface tension ~72mN/m*





# Inspiration on selection of Habbatus Sawda

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□ Al-Bukhari: Related by Aishah (Allah bless her) that the Prophet had said: the black seed cures everything except death.







# Inspiration on selection of Habbatus Sawda

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## Mentioned in Ancient Medical Manuscripts

Ibn Sina in  
Canon of  
Medicine

Sahih  
Bukhari vol. 7  
book 71, No  
592).

Tibb An-  
Nabawi  
Ibnu Al-  
Jauziyah

Kalonji is known around the world  
by many names because of its  
ancient popular history and  
medicinal value viz: black  
caraway, roman coriander, carvi  
(french), chwarzkummel (german),  
kalonji (hindi/urdu), kezah(hebrew)  
chernushka (russian), corek-out  
(turkish), habbat-albarakah or  
habbatus-  
sauda (Arabic) siyah daneh  
(Persian) etc.

Sahih  
Muslim: Book  
26 Kitab As-  
Salam,  
Number 5489

Old  
testament  
: Isaiah  
28:25,27

Tayyibul Ihsan  
Fi Tibb Al-Insan  
of Syeikh  
Ahmad Al-  
Fatani





# Inspiration on selection of Habbatus Sawda

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## Contemporary Medical Manuscripts

### Ingredients

### Quantity

Total oil 28-36%

Linoleic acid

40.3-58 %

Oleic acid

18-28 %

Steric acid

2.6-3.1 %

Palmitic acid

10-12%

Tocopherol (9-  
25mg/100g)

$\alpha$ -tocopherol

1.7-4 mg/100g

$\beta$ -tocopherol

4.9-18 mg/100g

$\gamma$ - tocopherol

1-18 mg/100g

Sterol (~2g/kg)

B -sitosterol

48-52%

5-avenasterol+  
campesterol+  
stigmasterol+  
10 more sterols

~50%

NIGELLA SATIVA oil anti inflammatory journal



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About 115,000 results (0.57 seconds)

A review on therapeutic potential of Nigella sativa: A miracle herb

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3642442/>

by A Ahmad - 2013 - Cited by 235 - Related articles

Mar 8, 2013 - Journal List - Asian Pac J Trop Biomed - v.3(5); 2013 May; PMC3642442 ... Keywords: Nigella sativa, Miracle herb, Ranunculaceae, ... It was showed that N. sativa seeds possess clinically useful anti H. pylori activity, comparable ... The antioxidant, anti-inflammatory, anticancer and antibacterial activities of ...

You visited this page on 4/4/17.

Anti-inflammatory effects of the Nigella sativa seed extract ... - NCBI - NIH

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4641935/>

by NI Chehl - 2009 - Cited by 145 - Related articles

We have recently shown that thymoquinone (Tq), the major constituent of Nigella sativa oil extract, induced apoptosis and inhibited proliferation in PDA cells.

You visited this page on 4/4/17.

The Protective Effects of Nigella sativa and Its Constituents on Induced ...

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4641935/>

by MR Khazdair - 2015 - Cited by 10 - Related articles

Oct 29, 2015 - The protective effect of N. sativa seeds against lead acetate-induced liver toxicity in .... H. Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. ... Journal of Taibah University Medical Sciences.

You visited this page on 4/4/17.

Nigella sativa as an anti-inflammatory and promising remyelinating ...

[www.sciencedirect.com/science/article/pii/S2090989614000265](http://www.sciencedirect.com/science/article/pii/S2090989614000265)

by HM Fahmy - 2014 - Cited by 5 - Related articles

Sep 26, 2014 - The Journal of Basic & Applied Zoology ... Nigella sativa as an anti-inflammatory and promising remyelinating agent in the cortex and ... In conclusion, N. sativa seeds could be used as a protective agent or an adjunct ...

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Inspiration on selection of  
Habbatus Sawda

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# Contemporary Medical Manuscripts

Other constituents:

Thymoquinone, nigellicine,  
nigellidine, flavonoids, tannins  
iron, calcium etc.



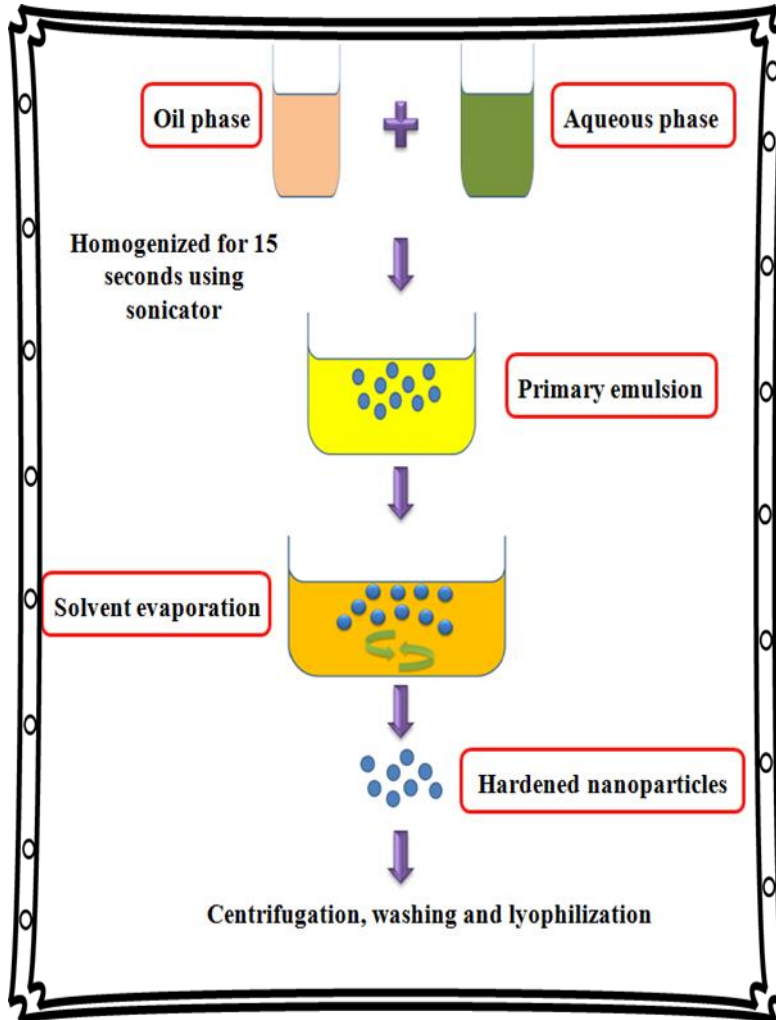
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# FABRICATION OF NANOPARTICLES

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OIL PHASE

1.2% w/v PLGA (IV 0.2 dL/g; 14 kDa)  
+ dichloromethane + ethyl acetate



1.2 % v/v Nigella sativa oil



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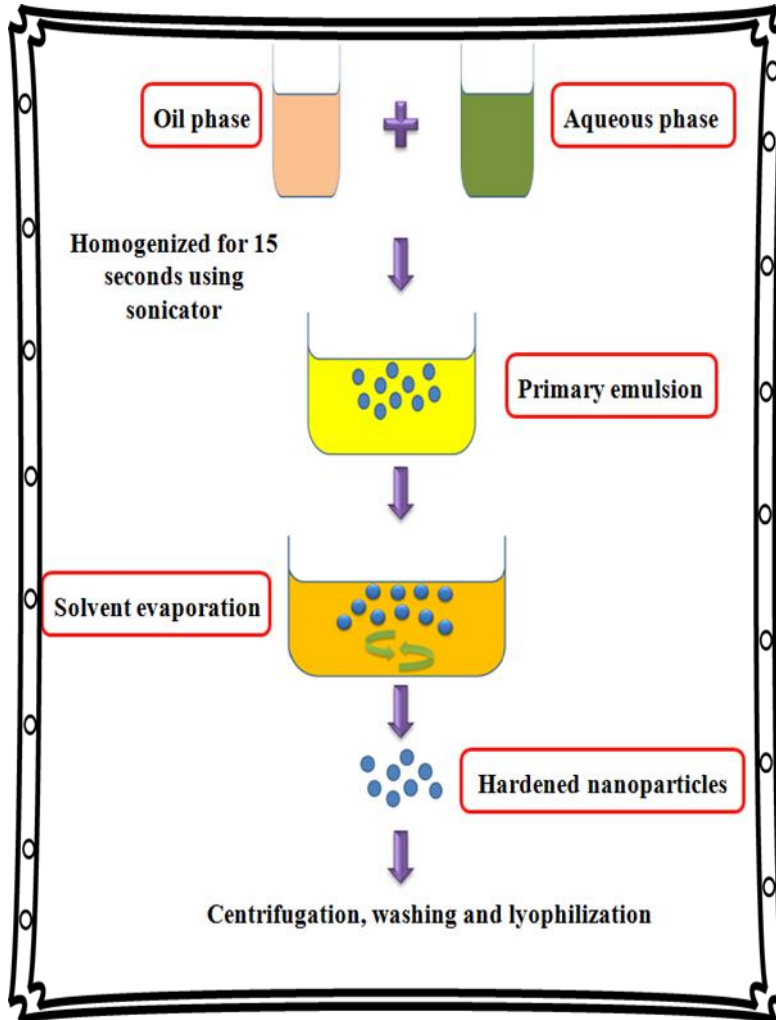
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# FABRICATION OF NANOPARTICLES

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## AQUEOUS PHASE

300  $\mu$ g plasmid DNA (*Gaussia* luciferase vector) + 30  $\mu$ l 2% CTAB

+ polyvinyl alcohol (PVA)

Low  
Molecular  
weight  
chitosan

Medium  
Molecular  
weight  
chitosan

Without  
chitosan



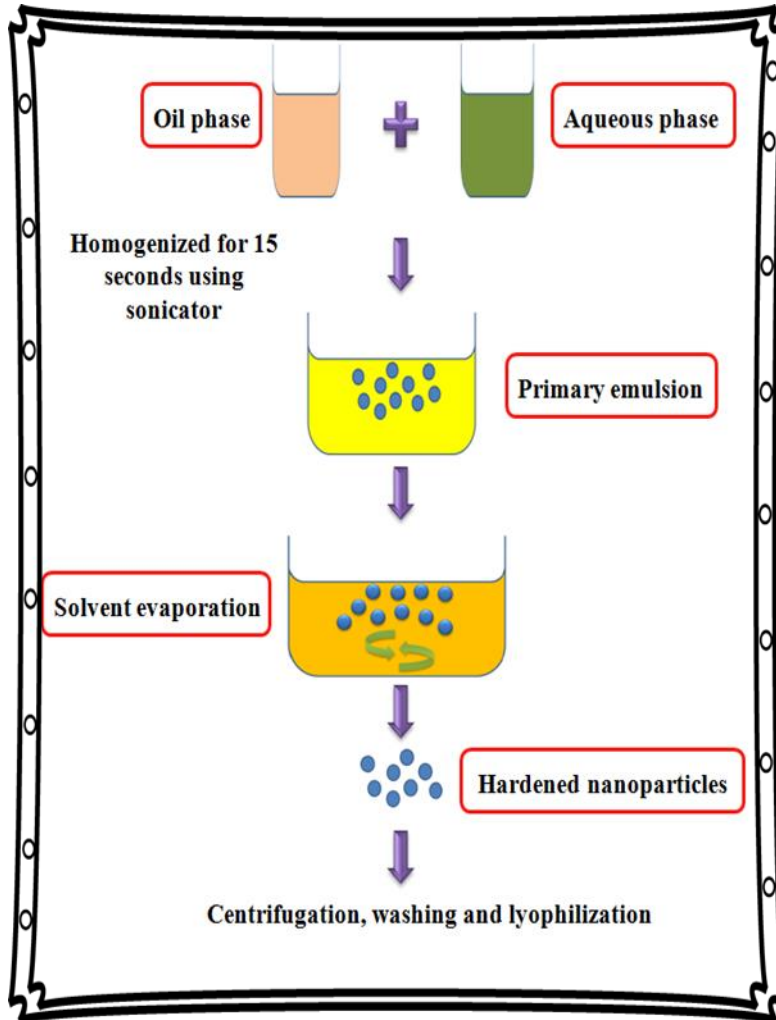
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# FABRICATION OF NANOPARTICLES

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HARDENING TANK

DEIONISED WATER +

Low  
Molecular  
weight  
chitosan

Medium  
Molecular  
weight  
chitosan

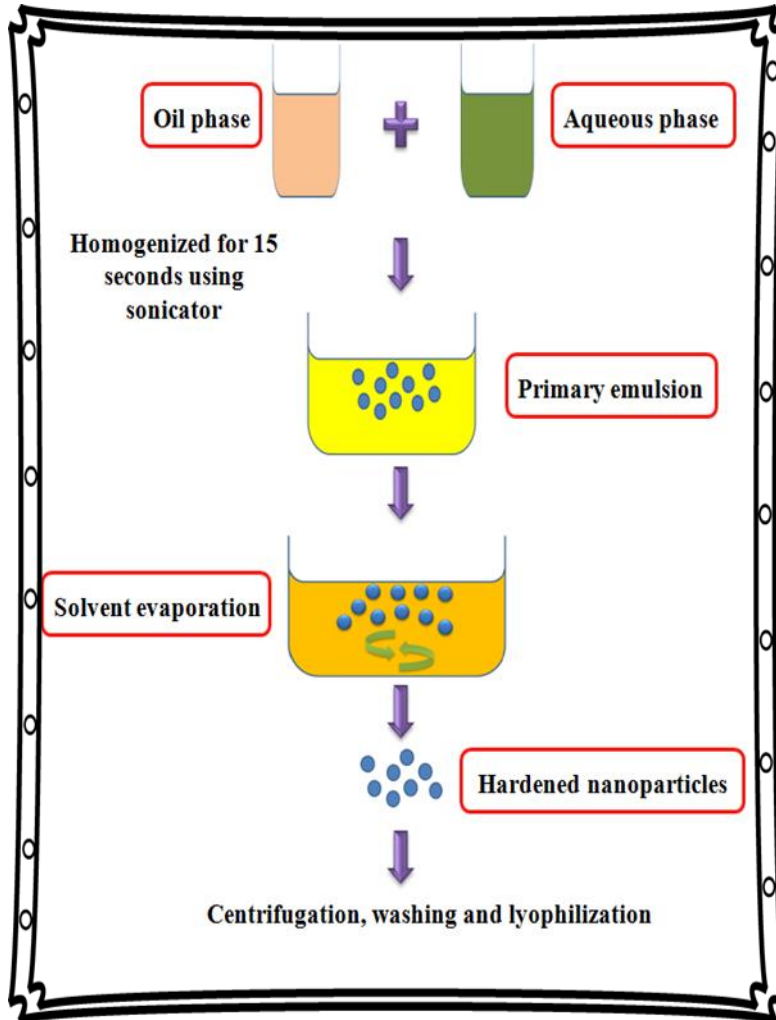
Without  
chitosan





# FABRICATION OF NANOPARTICLES

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VARYING PARAMETERS

AQUEOUS PHASE

Addition  
of Tween  
80, High  
or Low  
MW  
chitosan

HARDENING TANK





# FABRICATION OF NANOPARTICLES

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Constant processing method, varying the ingredients

No	Formulation	Ingredients					
		Chitosan	Polyvinyl alcohol	Polyethylene glycol	Hydroxyethyl methacrylate	Hydroxyethyl methacrylate	Hydroxyethyl methacrylate
1	Blank nanoparticle (.)	√	√	√			
2	Nanoparticle (.)	√	√	√	√		
3	Nanoparticle (.)	√	√	√	√	√	
4	Nanoparticle (.)	√	√	√	√		√
5	Nanoparticle (.)	√	√	√	√		√







# FABRICATION OF NANOPARTICLES

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Constant processing method, varying the ingredients

Phase	Ingredients	pDNA-loaded PLGA/N. sativa nanoparticle formulations				
		BN	NA	NB	NC	ND
Oil	14 kDa (0. 2A dL/g) PLGA (mg)	30	30	30	30	30
	Dichloromethane (ml)	0.5	0.5	0.5	0.5	0.5
	Ethyl Acetate (ml)	2	2	2	2	2
	pDNA-CTAB (mg)	0.3	0.3	0.3	0.3	0.3
	N. sativa Oil (ml)	-	0.03	0.03	0.03	0.03
Aqueous	PVA 1% w/v (ml)	3	3	3	3	3
	Deionized water (ml)	6	6	5.64	4.2	4.2
	Tween 80 1% (v/v) (ml)	-	-	0.36	-	-
	LMW chitosan 2% (w/v) (ml)	-	-	-	1.8	-
	MMW chitosan 2% (w/v) (ml)	-	-	-	-	1.8
Hardening tank	Deionized water (ml)	36	36	34.6	33	33
	Tween 80 1% (v/v) (ml)	-	-	1.4	-	-
	LMW chitosan 2% (w/v) (ml)	-	-	-	3	-
	MMW chitosan 2% (w/v) (ml)	-	-	-	-	3



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# FABRICATION OF NANOPARTICLES

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## Characterisation

Varying media for particle sizing

Dispersion	In water		Dispersion	In water		Dispersion	In water	
	Mean $\pm$ SD	SD		Mean $\pm$ SD	SD		Mean $\pm$ SD	SD
...	$\pm$ ..	..	...	$\pm$ ..	..	...	$\pm$ ..	..
...	$\pm$ ..	..	...	$\pm$ ..	..	...	$\pm$ ..	..
...	$\pm$ ..	..	...	$\pm$ ..	..	...	$\pm$ ..	..
...	$\pm$ ..	..	...	$\pm$ ..	..	...	$\pm$ ..	..
...	$\pm$ ..	..	...	$\pm$ ..	..	...	$\pm$ ..	..



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# FABRICATION OF NANOPARTICLES

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Characterisation

Surface morphology by Field  
Emission SEM

❖ **he**

❖ **...**

❖ **hitosan**



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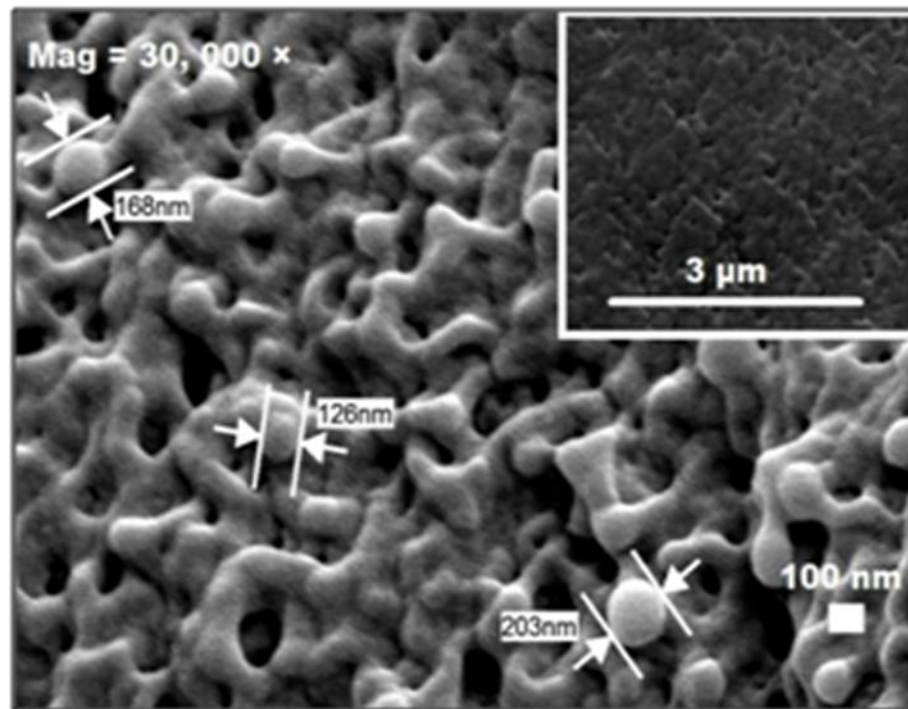
# FABRICATION OF NANOPARTICLES

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Characterisation

Surface morphology by Field Emission SEM



Blank Nanoparticles



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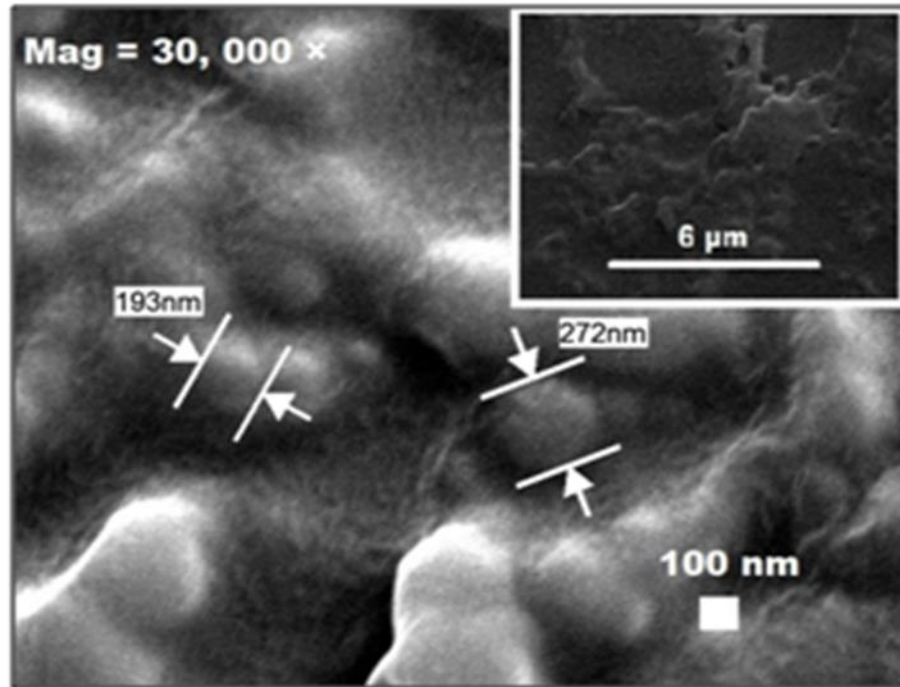
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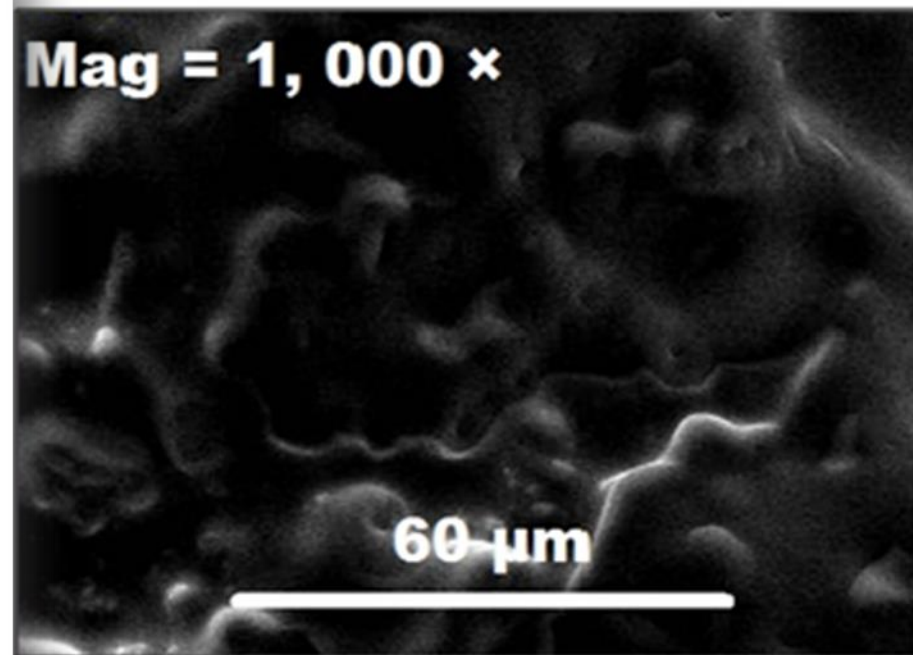


Characterisation

Surface morphology by Field Emission  
SEM



NA



NB



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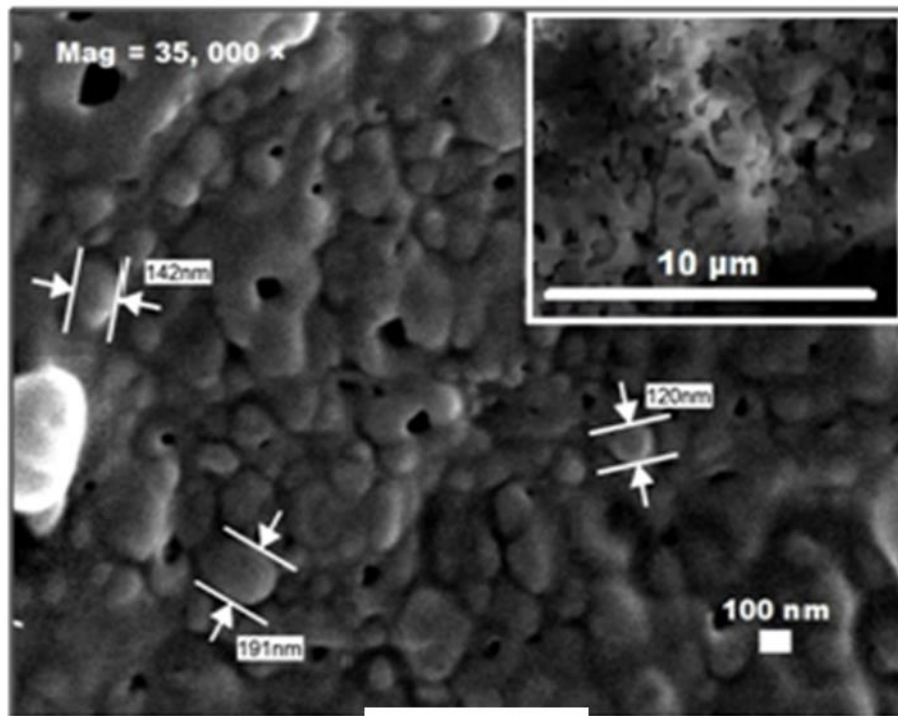
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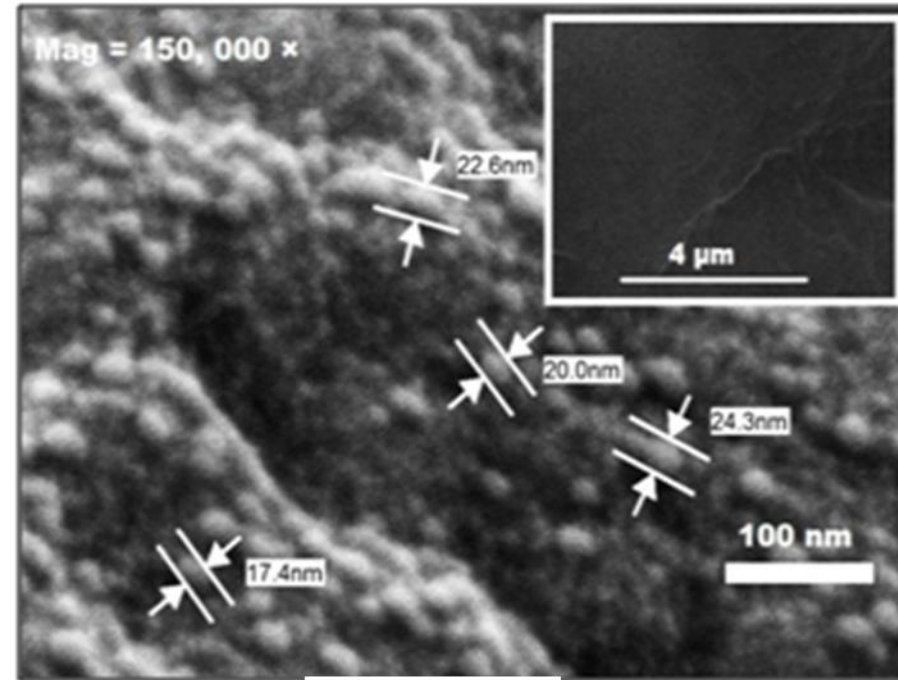


Characterisation

Surface morphology by Field Emission  
SEM



NC



ND



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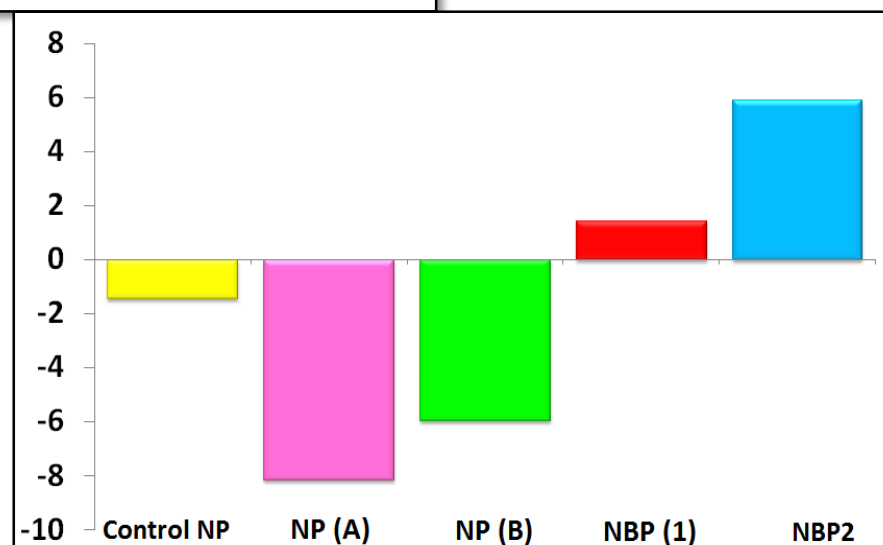
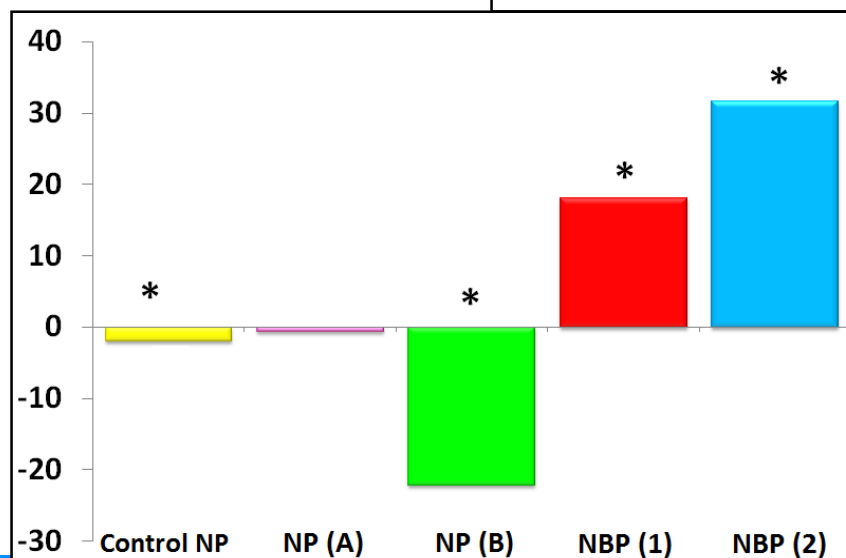
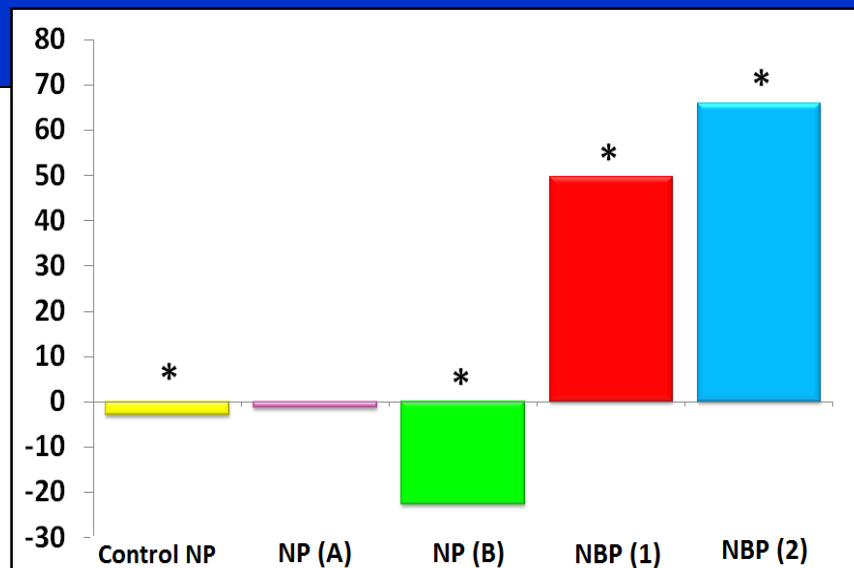
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## Characterisation

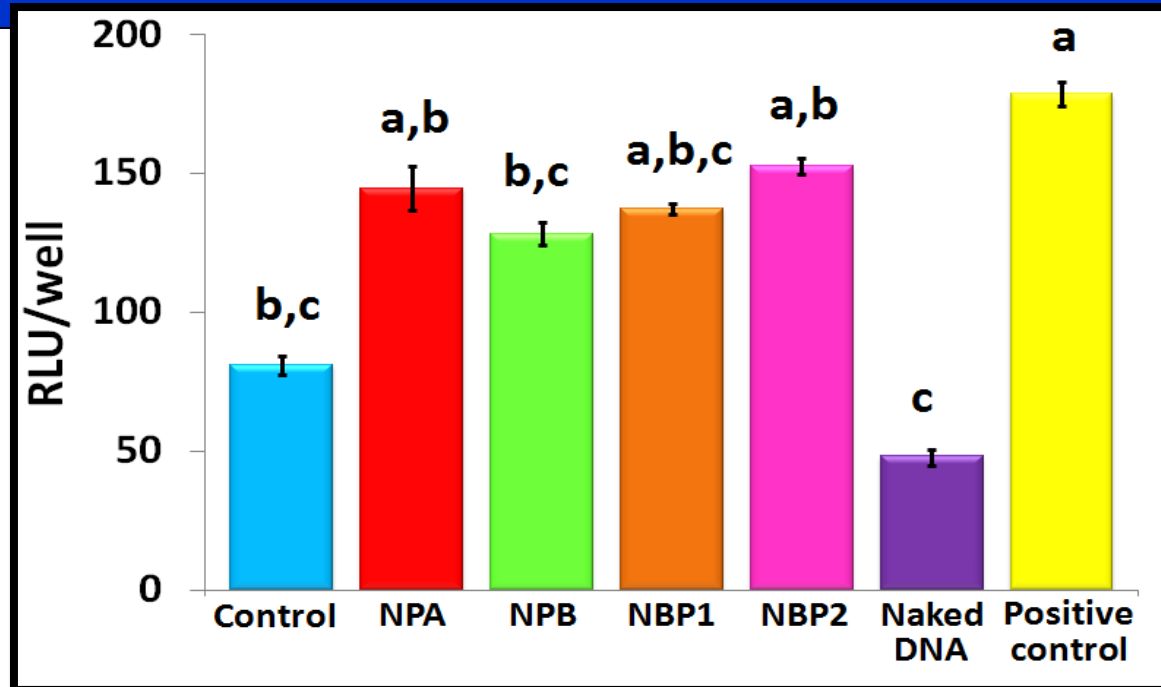
## Zeta potential

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lyophilized

suspended in the .



❖ The transfection could be seen in cells loaded with . . . and . . . having amount equivalent to .  $\mu\text{g}$  p . . . gave no significant difference compared to . . . a cells transfected with the positive control ( . . . enejuice: . . .  $\mu\text{L}$ : . . .  $\mu\text{g}$ ) and negative control ( . . .  $\mu\text{g}$  p . . .

❖ If all, the cells loaded with chitosan bearing nanoparticle, . . . which consisted of medium molecular weight of chitosan successfully transfected . . . a cells and gave pronounced gene expression.







## Characterisation

# *N.Sativa* effect on neurite outgrowth of Neuro2a cells

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sample	conc. ( $\mu\text{g/ml}$ )	length ( $\mu\text{m}$ )			
		..	..	..	..
control	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
..0	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
..0	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
..0	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
..0	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..
	.	$\pm$ ..	$\pm$ ..	$\pm$ ..	$\pm$ ..



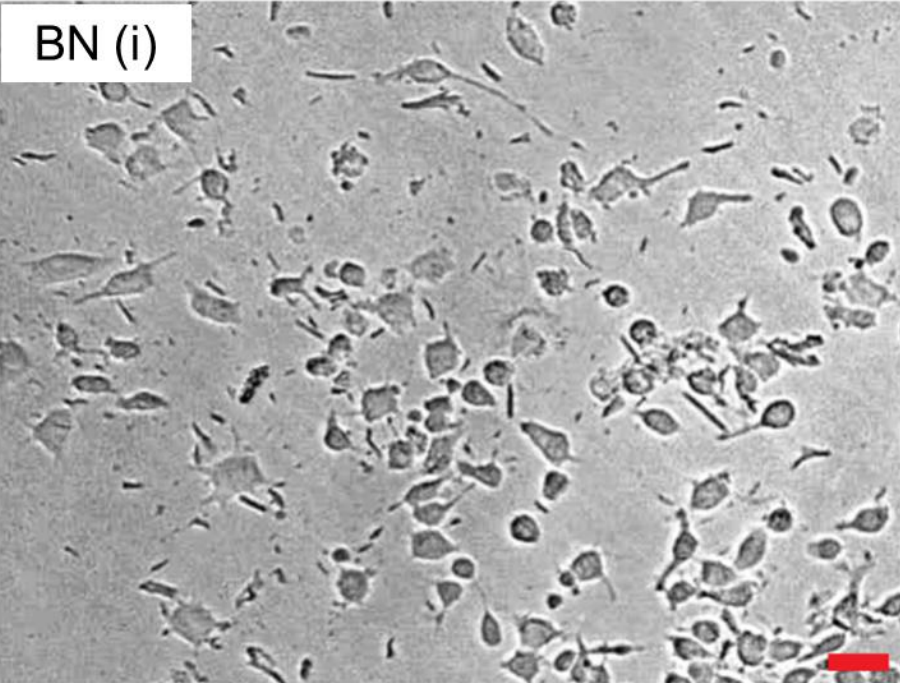
Characterisation

# *N. Sativa* effect on neurite outgrowth of Neuro2a cells

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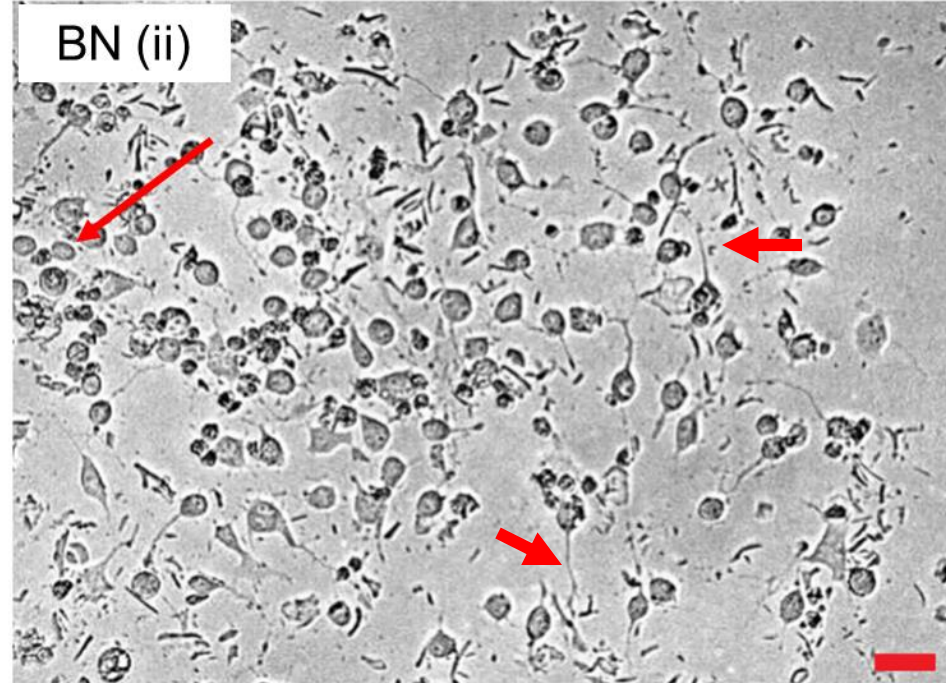


BN (i)



100 µg/ml of BN incubated for 24h

BN (ii)



100 µg/ml of BN incubated for 96h



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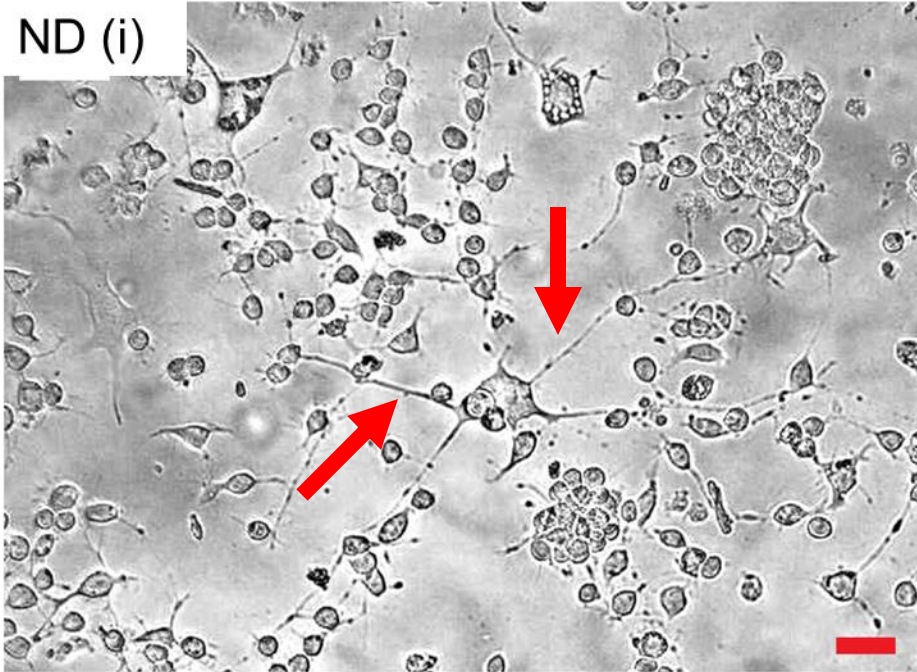
Characterisation

# *N. Sativa* effect on neurite outgrowth of Neuro2a cells

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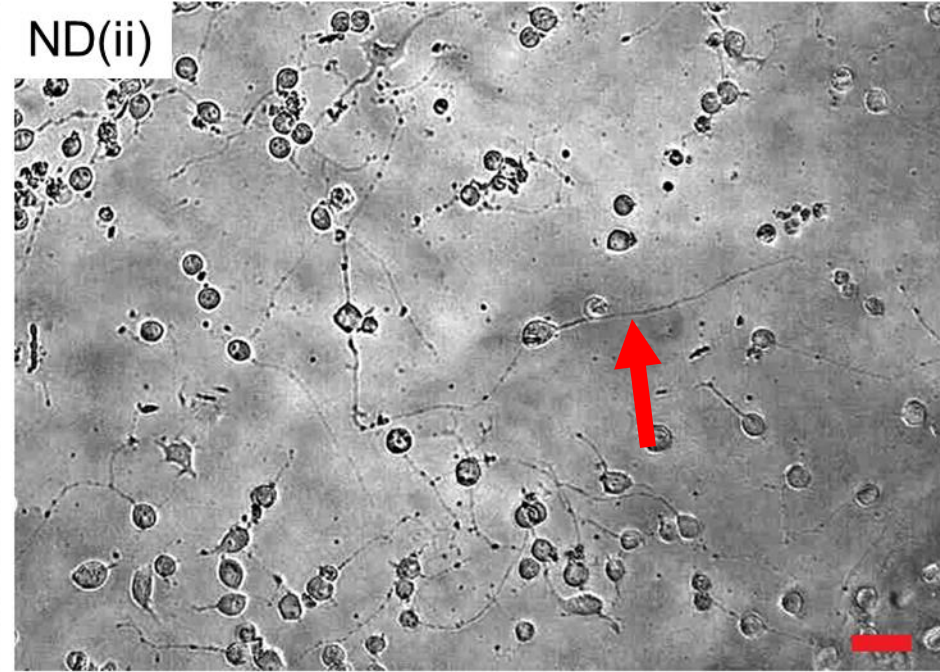


ND (i)



100 µg/ml of ND incubated for 24h

ND(ii)



100 µg/ml of ND incubated for 96h



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### Summary of Discussion & Conclusion

- ❖ Plasmid DNA was successfully co-encapsulated with *N.sativa* oil as nanoparticles
- ❖ This nanoparticles displayed ability to transfect (indicated pDNA remain functional) and able to induce neurite outgrowth (exerted by *N.sativa*) → probably potentially be used as neuroregenerative drug
- ❖ The fusion between contemporary medicine which is water-soluble (pDNA) and oily natural product cum prophetic medicine (*N.sativa*) showed enhanced gene transfection with another attribute i.e neuroregeneration







Strategy #2

Fabrication of Nanoemulsion

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# Fabrication of Doxycycline + Nigella sativa Nanoemulsion



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## Physicochemical properties

# Doxycycline

- Small molecular weight
- Water-soluble / hydrophilic
- Light-sensitive
- Commercially available in powder





## Physicochemical properties **Nigella sativa oil**

- Has multiple compounds (~50% fatty acids + ~40% sterols + 10% tocopherol)
- Lipophilic / oil
- Soluble in organic solvent
- Surface tension ~30mN/m

*Water surface tension ~72mN/m*





## Physicochemical properties **Lecithin**

- a phospholipid molecule (phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol)
- <36% Fats+fixed oil







## Strategy #2

# Fabrication of Nanoemulsion

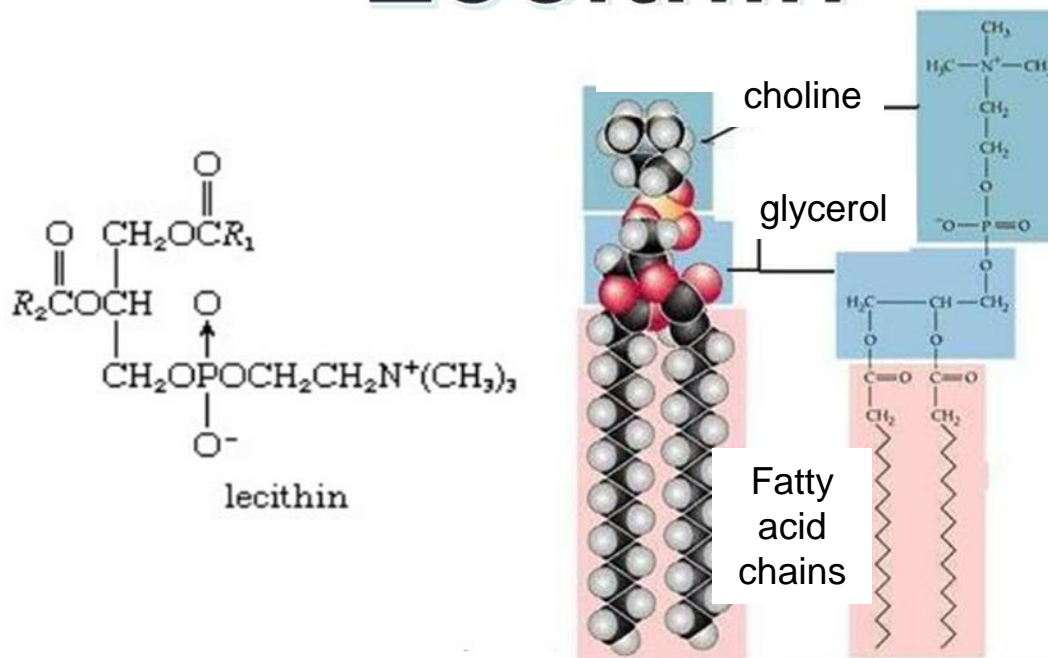
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## Physicochemical properties

### Lecithin

- Composed of phosphoric acid, cholines, esters of glycerol, and two fatty acids
- The term 'lecithin' is also used for a mixture of phosphoglycerides containing principally lecithin and cephalin (phosphatidylethanol amine, phosphatidylinositol)



A lecithin molecule has a nonpolar portion (fatty acid ester gp) and polar portion (phosphorylcholine) → amphiphilic molecule





Strategy #2

# Fabrication of Nanoemulsion

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## Preparation method: **Aqueous Phase**

- Doxycycline
- Preservatives
- Dissolve using pneumatic stirrer



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Strategy #2

# Fabrication of Nanoemulsion

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## Preparation method: **Oil Phase**

- Nigella sativa oil
- Eugenol
- Mix using pneumatic stirrer



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Strategy #2

Fabrication of Nanoemulsion

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## Preparation method: **Nanoemulsifiers Phase**

- Lecithin
- Co-surfactants + water
- Pulsative mixing using agitator  
(10, 20, 10 mins; 50 rpm)



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## Preparation method: Nanoemulsification

- Aqueous and oil phases added into nanoemulsifier phase
- Combined technique of agitator (30rpm) + homogenizer (3000rpm) for 30min
- Equipment: Vacuum Homogeniser





# Strategy #2 Fabrication of Nanoemulsion

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## Preparation method: Nanoemulsification



Vacuum Homogeneous Mixer



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## Strategy #2

# Fabrication of Nanoemulsion

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Characterisation

Droplet size, PDI, Zeta potential

Formula	Droplet size (nm)	PDI	Zeta potential (mV)
19% lecithin	288	0.427	-69.2
23% lecithin	235	0.389	-63.3







## Strategy #2

# Fabrication of Nanoemulsion

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### Characterisation

19% Lecithin

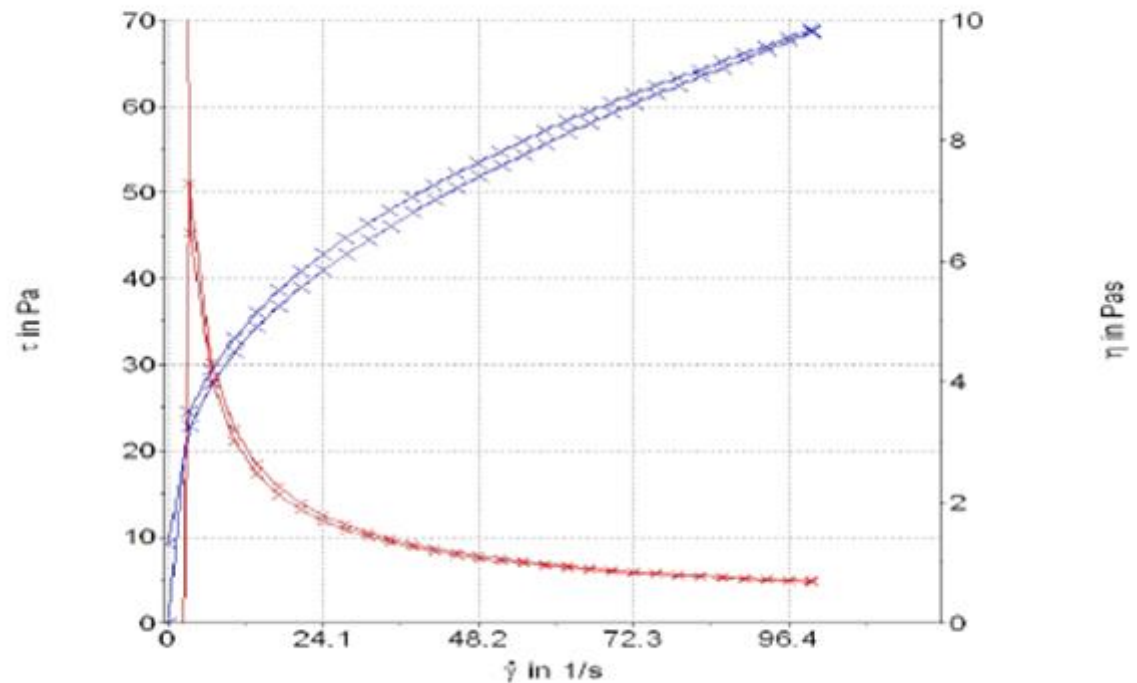
Viscosity 3.22 Pa.s

Thixotropy 124.4 Pa.s

**Thixotropy** – broken down  
with time when sheared;  
rebuilds when left to rest

### Rheological properties

20L\_30min  
—  $\tau = f(\dot{\gamma})$   
—  $\eta = f(\dot{\gamma})$







## Strategy #2

# Fabrication of Nanoemulsion

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### Characterisation

#### 23% Lecithin

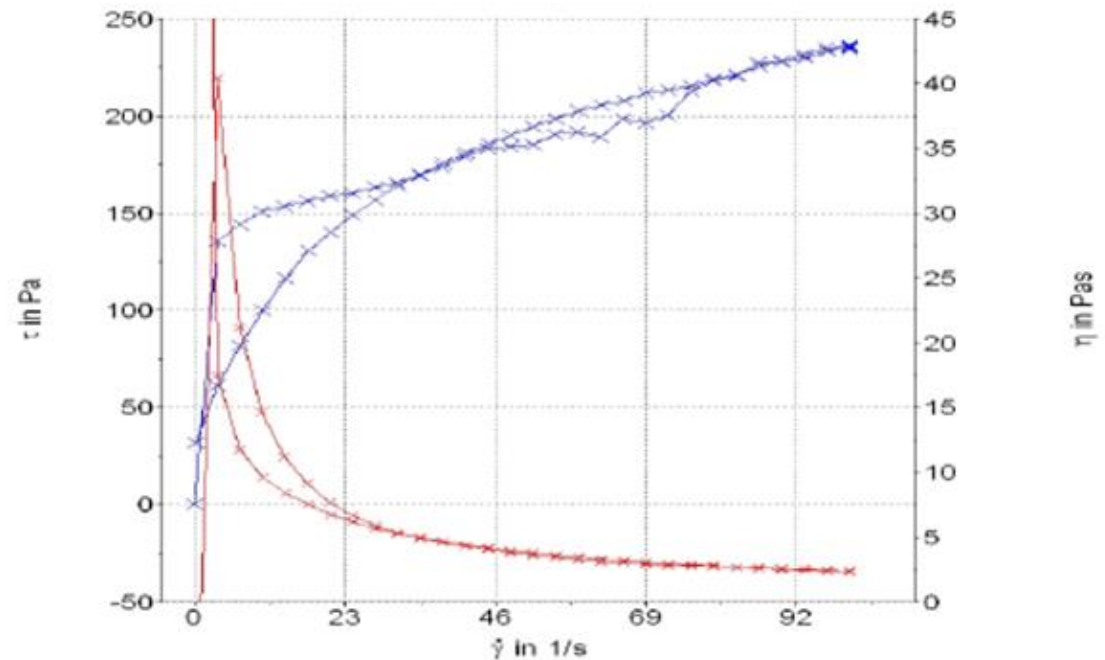
Viscosity 14.68 Pa.s

Thixotropy 650.2 Pa.s

**Thixotropy** – broken down  
with time when sheared;  
rebuilds when left to rest

### Rheological properties

periodox\_scaleup\_23%  
 $\tau = f(\dot{\gamma})$   
 $\eta = f(\dot{\gamma})$





## Strategy #2

# Fabrication of Nanoemulsion

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### Summary of Discussion & Conclusion

- ❖ Due to amphiphilic structure, lecithin with the addition of suitable co-surfactants can yield a stable nanoemulsion
- ❖ Nanoemulsion of doxycycline + *Nigella sativa* oil displayed a non-Newtonian, thixotropic fluid → suitable for intended use as locally applied antibiotic for periodontitis
- ❖ The fusion between contemporary medicine which is water-soluble API (doxycycline) and oily natural product cum prophetic medicine (*N.sativa*) is postulated to improve doxycycline efficacy due to improved 'BCS' classes, from BCS Class III to BCS Class I





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